WHAT IS CLAIMED IS:

1. A method for site-directed mutagenesis comprising the steps of:

providing two terminal tailed primers comprising an anchor portion and a portion having nucleotide sequences respectively complementary to each end of a gene to be mutated;

annealing the complementary portion of said terminal tailed primers and a set of mutagenic primers to template DNA in a single step;

synthesizing a mutant strand by primer extension and ligation; and amplifying said mutant strand by polymerase chain reaction.

- 2. The method of claim 1, wherein said method is used to mutate 5 nucleotides simultaneously.
- 3. The method of claim 1, wherein said method is used to mutate 10 nucleotides simultaneously.
- 4. The method of claim 1, wherein said method is used to mutate more than 10 nucleotides simultaneously.
- 5. The method of claim 1, wherein said method is used to mutate 20 nucleotides simultaneously.
- 6. The method of claim 1, wherein said method is used to mutate 30 nucleotides simultaneously.
- 7. The method of claim 1, wherein said method is used to mutate 40 nucleotides simultaneously.
- 8. The method of claim 1, wherein said method is used to mutate 50 nucleotides simultaneously.
- 9. The method of claim 1, wherein said method is used to mutate more than 50 nucleotides simultaneously.
- 10. The method of claim 1, wherein said complementary nucleotide sequences of said terminal tailed primers are 25 nucleotides in length.
- 11. The method of claim 1, wherein said complementary nucleotide sequences of said terminal tailed primers are less than 25 nucleotides in length.
- 12. The method of claim 1, wherein said complementary nucleotide sequences of said terminal tailed primers are more than 25 nucleotides in length.

- 13. The method of claim 1, wherein each said anchor portion of said terminal tailed primers comprise at least one restriction endonuclease site.
- 14. The method of claim 1, wherein each said anchor portion of said terminal tailed primers comprise at least three restriction endonuclease sites.
- 15. The method of claim 1, wherein said set of mutagenic primers comprises one mutagenic primer for each desired mutation.
- 16. The method of claim 1, wherein said set of mutagenic primers comprises one mutagenic primer for two or more desired mutations.
- 17. The method of claim 1, wherein said two or more desired mutations are located less than 25 nucleotides apart.
- 18. The method of claim 1, wherein said primer extension is performed using a DNA polymerase.
- 19. The method of claim 18, wherein said DNA polymerase comprises T4 DNA polymerase.
- 20. The method of claim 18, wherein said DNA polymerase comprises T7 DNA polymerase.
- 21. The method of claim 18, wherein said DNA polymerase comprises *E.coli* DNA polymerase I.
- 22. The method of claim 18, wherein said DNA polymerase comprises the Klenow fragment of DNA polymerase I.
- 23. The method of claim 18, wherein said DNA polymerase comprises Moloney Murine Leukemia Virus reverse transcriptase.
- 24. The method of claim 18, wherein said DNA polymerase comprises *Pfu* DNA polymerase.
- 25. The method of claim 18, wherein said DNA polymerase comprises *Tli* DNA polymerase.
- 26. The method of claim 18, wherein said DNA polymerase comprises *Bst* DNA polymerase.
- 27. The method of claim 18, wherein said DNA polymerase comprises *Taq* DNA polymerase.
- 28. The method of claim 18, wherein said DNA polymerase comprises *Pwo* DNA polymerase.
- 29. The method of claim 18, wherein said DNA polymerase comprises *Tth* DNA polymerase.

- 30. The method of claim 18, wherein said DNA polymerase comprises *Tfl* DNA polymerase.
- 31. The method of claim 1, wherein said ligation is performed using a DNA ligase.
- 32. The method of claim 31, wherein said ligase comprises T4 DNA ligase.
- 33. The method of claim 31, wherein said ligase comprises T7 DNA ligase.
- 34. The method of claim 31, wherein said ligase comprises *Pfu* DNA ligase.
- 35. The method of claim 31, wherein said ligase comprises *Tth* DNA ligase.
- 36. The method of claim 31, wherein said ligase comprises *Tsc* DNA ligase.
- 37. The method of claim 31, wherein said ligase comprises *Taq* DNA ligase.
- 38. The method of claim 31, wherein said ligase comprises an NAD-dependent DNA ligase.
- 39. The method of claim 1, wherein said polymerase chain reaction is performed using a DNA polymerase.
- 40. The method of claim 39, wherein said DNA polymerase comprises *Pfu* DNA polymerase.
- 41. The method of claim 39, wherein said DNA polymerase comprises *Pwo* DNA polymerase.

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- 42. The method of claim 39, wherein said DNA polymerase comprises *Tli* DNA polymerase.
- 43. The method of claim 39, wherein said DNA polymerase comprises *Tth* DNA polymerase.
- 44. The method of claim 39, wherein said DNA polymerase comprises *Tfl* DNA polymerase.
- 45. The method of claim 1, wherein said polymerase chain reaction is performed using a DNA polymerase blend comprising at least two DNA polymerases.
- 46. The method of claim 45, wherein said DNA polymerase blend comprises *Taq* DNA polymerase and a proofreading DNA polymerase.
- 47. The method of claim 1, wherein said template DNA is double-stranded DNA.
- 48. The method of claim 1, wherein said template DNA is single-stranded DNA.
- 49. The method of claim 1, wherein said mutagenic primers comprise a three-fold molar excess over said terminal tailed primers.
- 50. The method of claim 1, wherein said mutagenic primers each have a G or C at their 3' ends at the location at which DNA synthesis is initiated.
- 51. The method of claim 1, wherein said mutagenic primers are 5'-phosphorylated.

52. The method of claim 1, wherein said terminal tailed primers are 5'-phosphorylated.

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